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(54) METHOD FOR DETERMINING SUGAR CHAIN STRUCTURE

(57)Abstract:
PURPOSE: To simply and efficiently determine a sugar chain structure by fixing the sugar chain or sugar peptide derived from composite carbohydrate to a solid phase, binding a marker material to it, and detecting the marked solid phase.
CONSTITUTION: The composite carbohydrate such as sugar protein or glycolipid isolated from cells or a living sample derived from the cells is digested by enzyme to obtain the sugar chain or sugar peptide, and it is reacted with a solid phase (resins, celluloses, glass or the like) coated with a polymer having the reaction group (epoxy group, halogen atom or the like) capable of being reacted with the molecular terminal group of them and forming a covalent bond to be fixed. A marker material (e.g. marked lectin, marked antibody) peculiarly bonded with them is bonded, the marked solid phase is detected, or a bonding material (unmarked lectin and antibody or the like) peculiarly bonded with the sugar chain or sugar peptide is reacted, a material peculiarly bonded with the bonding material is reacted, and the generated marked solid phase is detected.

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CLAIMS

[Claim(s)]
[Claim 1] (1) Make the solid phase coated with the polymer which has the reaction radical which reacts with the molecule end group of the sugar chain of the glycoconjugate origin or glycopeptide, and this sugar chain or this glycopeptide, and can form covalent bond react, and generate a fixed sugar chain or fixed glycopeptide. (2) How to determine the sugar chain structure characterized by detecting the labeled solid phase which this fixed sugar chain or fixed glycopeptide, and the labeled matter that is specifically combined with these are made to react, and carries out (3) generation.
[Claim 2] (1) Make the solid phase coated with the polymer which has the reaction radical which reacts with the molecule end group of the sugar chain of the glycoconjugate origin or glycopeptide, and this sugar chain or this glycopeptide, and can form covalent bond react, and generate a fixed sugar chain or fixed glycopeptide. (2) This fixed sugar chain or fixed glycopeptide, and the matter for association specifically combined with these are made to react. (3) The product obtained, How to determine the sugar chain structure characterized by detecting the labeled solid phase which the marker specifically combined with this matter for association is made to react, and carries out (4) generation.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the approach of determining efficiently the sugar chain structure of glycoconjugate, such as a glycoprotein, a glycolipid, and proteoglycan, simple, in more detail about the approach of determining sugar chain structure.
[0002]
[Description of the Prior Art] The sugar chain of glycoconjugate widely distributed over the nature is an important constituent in the living body, and it is shown clearly that it is deeply concerned with the interaction between cells. In connection with it, the technique of the minute amount analysis of various sugar chain structures is developed, although these techniques combine suitably the process of logging of a sugar chain, separation purification of a sugar chain, the indicator of a sugar chain, etc., in order to acquire the positive information about the obtained sugar finally, identification by NMR or MS is required for them, and they require very complicated time and effort. The method of saving such time and effort and determining sugar chain structure simple is also proposed.
[0003] The simple sugar chain structural-analysis approach proposed until now for example, as a thing adapting the technique of high performance chromatography (HPLC) The analytical method using the column which fixed the lectin which has sugar discernment ability (Anal.Biochem., 164, 374 (1987) Harada et al.), The approach by 2-dimensional HPLC (Anal.Biochem., 171, 73 (1988) TOMIYA et al.) which combined HPLC in two kinds of modes, And a sample can be beforehand processed in mixed enzyme system trains, such as exoglycosidase, and the approach (chemistry and living thing 32 (10) 661 (1994) Konishi et al.) of determining sugar chain structure etc. can be mentioned by analyzing the processed sample by HPLC. Each of these is the approaches of determining sugar chain structure for the holding time of the chromatogram of HPLC as an index.
[0004] However, the analytical method using the technique of HPLC (1) Only analysis

of one sample can be performed at once and many samples cannot be analyzed to coincidence. (2) with the delicate conditioning of HPLC, and high possibility of the holding time shifting and becoming incorrectness (3) If (4) lectin fixed column which consumes a reagent in large quantities is used, what combined HPLC with the postcolumn reactor or the precolumn reactor Troubles, like there is possibility that effect will arise also in the glycopeptide to which it sticks for change of the compatibility over the sugar of lectin are held.

[0005] Moreover, although the method of fixing and analyzing a sugar chain to solid phase is also tried, fixing the high sugar chain of a hydrophilic property is technically accompanied by difficulty. Therefore, the technique for fixing a sugar chain is proposed. For example, the approach (Anal.Biochem., 182, 200 (1989) OOBAYASHI et al.) of fixing by acid-amide association on an amino plate using the reducing terminal of a sugar chain, the method (JP,62-212568,A) of giant-molecule-izing in order to give hydrophobicity to the sugar chain itself, and making it stick to a plate, and a sugar chain are BIOCHINIRU-ized, and the approach of fixing a sugar chain in the solid phase which combined avidin using the powerful compatibility of avidin and a biotin etc. is mentioned. However, in the approach of fixing in the solid phase of a plate etc., there were troubles -- decline in the immobilization yield accompanying pretreatment of a sugar chain and solid-phase-izing using a condensing agent, the complicatedness of actuation, and long duration are required, and it is easy to produce the nonspecific adsorption by making a biogenic substance live together etc.. Moreover, although analyzing a sugar chain by the tools of analysis using surface plasmon resonance recently is also proposed, in order to use an expensive special device, it is not the approach of being rich in versatility.

[0006]

[Problem(s) to be Solved by the Invention] The purpose of this invention is by fixing efficiently the sugar chain or glycopeptide originating in glycoconjugate in solid phase by the single step, and detecting the labeled solid phase which fixes a marker on it and is acquired further to offer the approach that sugar chain structure can be determined efficiently simple. According to the approach of this invention, a series of necessary reactions can be efficiently carried out to analysis using the conventional detection device, and many micro samples can be analyzed at once.

[0007]

[Means for Solving the Problem] According to this invention, make the solid phase coated with the polymer which has the reaction radical which reacts with the molecule end group of the sugar chain of (1) glycoconjugate origin or glycopeptide, and this sugar chain or this glycopeptide, and can form covalent bond react, and a fixed sugar chain or fixed glycopeptide is generated. (2) This fixed sugar chain or fixed glycopeptide, and the labeled matter that is specifically combined with these are made to react, and the method of determining the sugar chain structure characterized by detecting the labeled solid phase which carries out (3) generation is offered.

[0008] Furthermore, according to this invention, the sugar chain or glycopeptide of (1)

glycoconjugate origin, Make the solid phase coated with the polymer which has the reaction radical which reacts with the molecule end group of this sugar chain or this glycopeptide, and can form covalent bond react, and a fixed sugar chain or fixed glycopeptide is generated. A fixed (2) this sugar chain or fixed glycopeptide, and the matter for association specifically combined with these are made to react. (3) The product obtained, The marker specifically combined with this matter for association is made to react, and the method of determining the sugar chain structure characterized by detecting the labeled solid phase which carries out (4) generation is offered.
[0009] Hereafter, how to determine the sugar chain structure of this invention is explained to a detail. The measuring object of this invention is the sugar chain structure of the sugar containing nature components of nonsugar, such as glycoconjugate, i.e., a glycoprotein, a glycolipid, and proteoglycan, any of a monosaccharide, an oligosaccharide, and a polysaccharide are sufficient as sugar itself, and it is not limited exceptionally.
[0010] The specimen to be used is not limited exceptionally and sugar chains, such as various kinds of things, for example, N-glycoside mold, O-glycoside mold, a collagen mold glycoprotein and a gun griot system, a GUROBO system, and a RAKUTO system glycolipid, are mentioned as a sugar chain contained in a specimen. As an approach of separating a sugar chain from glycoconjugate, such as a glycoprotein, a glycolipid, and proteoglycan, the decomposing method by the thorough digestion by the approach used regularly, for example, a hydrazinolysis method, and pronase, N-glycanase processing, O-glycanase processing, and trifluoroacetic acid etc. can be mentioned, and processing actuation by them etc. can be performed like what is taken by this usual kind of approach. (Methods Enzymol., Vol 83, 263(1982))
[0011] In the approach of this invention, the solid phase which fixed the sugar chain or **** PUCHIDO of the glycoconjugate origin is prepared first. For that purpose, digestion according glycoconjugate, such as a glycoprotein first isolated from the living thing preparation originating in a cell or it, a glycolipid, and proteoglycan, to an enzyme etc. is performed, and the fragmentation of a sugar chain or **** PUCHIDO is obtained. By HPLC etc., each fragmentation carries out separation purification and obtains optimum dose picking, the sugar chain for immobilization, or the fragmentation of **** PUCHIDO for each peak fraction. Although various chromatographies can be used for the fractionation of a sugar chain, the opposition HPLC by C18 grade is suitable for separation of low-molecular matter like especially **** PUCHIDO. It is simple on an elution fraction monitor to detect absorption of 210 to 230 nm, and it is good for him. The obtained fraction carries out preparative isolation desiccation for every peak, and is used for a reaction with the solid phase for immobilization.
[0012] The solid phase which should fix the fragmentation of a sugar chain or **** PUCHIDO is not limited exceptionally, and a poorly soluble or insoluble synthetic polymer, celluloses, glass, etc. are mentioned to water, such as fluorination polymers, such as polyolefine, polystyrene, permutation polystyrene, polytetrafluoroethylene, and polyvinylidene fluoride, polysulfone, polyester, a polycarbonate, a polyacrylonitrile,

nylon, polyacrylamide, polymethacrylate, polyvinyl alcohol, polyurethane, and these copolymers, as the quality of the material. It is not limited exceptionally, a microplate, the film, fiber, paper, etc. are mentioned as a typical gestalt, and the gestalt of solid phase also has especially the most desirable microtiter plate. Moreover, the magnitude and thickness are not limited exceptionally, either.

[0013] In order to face fixing on a sugar chain or the solid phase of **** PUCHIDO and to heighten bonding strength, it coats with the polymer which has beforehand the reaction radical which can form covalent bond (for example, acid-amide association) for a solid phase front face with a sugar chain or the molecule end group of **** PUCHIDO, i.e., the formyl group of the reducing terminal of a sugar chain, the amino group of the molecule end of **** PUCHIDO, or a carboxyl group. As a reaction radical which can form such covalent bond, an epoxy group, a halogen atom, the amino group, a hydrazino radical, a carboxyl group, an acid-anhydride radical, a formyl group, etc. are mentioned, for example.

[0014] The polymer which has the above reaction radicals is prepared by the chemical denaturation of the polymer which has the polymerization and other reaction radicals of a monomer which have the above reaction radicals etc. As a desirable polymer, a maleic-anhydride copolymer with the polymer of an acid-anhydride monomer like a maleic anhydride or it and the vinyl monomer which may be copolymerized, for example, the methyl vinyl ether, ethyl vinyl ether, butyl vinyl ether, isobutyl vinyl ether, styrene, etc. can be illustrated. Furthermore, the polymer and copolymers which are the monomers which have the glycidyl methacrylate which is the monomer which has an epoxy group, glycidyl acrylate, or a carboxyl group, such as an acrylic acid and a methacrylic acid, can be mentioned. It is also possible to obtain the polymer which has a functional group which carries out chemistry denaturation of the functional group of the obtained polymer further, and is different, for example, the polymer which has an epoxy group or an acid anhydride can obtain the polymer which has an amino group by making it react with ammonia, a hydrazine or the diamino alkanes like propanediamine, basic amino acid, and polyamine. Moreover, the polymer which has an amino group can be used as the polymer which has a carboxyl group by making it react with an acid anhydride like a succinic anhydride etc. For example, under alkali conditions, it can be made to be able to react with the diepoxide like diglycidyl ether [like 1,4-butanediol diglycidyl ether], 1, and 7-OKUTA diene diepoxide in the case of the polymer which has a hydroxyl group, and can consider as the polymer which has an epoxy group.

[0015] Although especially the molecular weight of the polymer for these covering is not restricted, in order to have the hydrophobicity which was suitable for the solid phase front face carrying out a coat, and viscosity, as for molecular weight, 1 million especially or less [20,000 or more] are [that what is necessary is just 400 or more] desirable. The approach of carrying out the coat of the polymer which has the above-mentioned reaction radical to solid phase is not limited exceptionally, and can take the coating method of dip coating, the applying method, and others for the

organic solvent solution of a polymer.
[0016] Especially the reaction condition of a sugar chain, or the fraction of **** PUCHIDO and fixed solid phase is not restricted, is usually the same as that of the case of this kind of the detecting method, and is good. Namely, generally 45 degrees C or less react by requiring about 1 - 40 hours under an about 4-40-degree C temperature condition preferably. As a reaction medium, the with a pH [of the usual solvent which does not have a bad influence on a reaction, for example, citrate buffer solution, a phosphate buffer solution, the tris-hydrochloric-acid buffer solution the acetic-acid buffer solution, etc.] of about four to eight buffer solution can be used as a desirable thing in that case.
[0017] (1) this sugar chain or **** PUCHIDO after fixing on a sugar chain or the solid phase of **** PUCHIDO as mentioned above in the approach of this invention, After making the labeled matter which is specifically combined with these react and joining together, detect the labeled solid phase to generate, or Or (2) this sugar chain or **** PUCHIDO, After making the matter for association specifically combined with these react, making the marker further combined with this matter for association specifically react and joining together, the labeled solid phase to generate is detected.

[0018] In the approach of the above (1), indicator lectin and a labelled antibody are preferably used as labeled matter which is specifically combined with the fixed sugar chain or **** PUCHIDO. As indicator lectin and a labelled antibody, that which labeled various kinds of lectins and antibodies by various indicator agents, such as the usual radioactive substance, an enzyme marker, and a fluorescent material, is used here, respectively. As the radioactive substance as an indicator agent, the radioactive iodine agent of 125I and others etc. as a fluorescent material Fluorescein isothiocyanate (FITC), tetramethyl rhodamine isothiocyanate (TRITC), Permutation rhodamine isothiocyanate (XRITC), rhodamine B and isothiocyanate, a dichloro triazine fluorescein (DTAF) etc. -- moreover, as an enzyme marker A par oxidase, a micro par oxidase, chymotrypsinogen, procarboxypeptidase, glycero aldehyde-3-phosphoric-acid dehydrogenase, an amylase, a phosphorylase, DNA-NAZE, the beta-galactosidase, etc. can be mentioned, respectively.
[0019] What [various kinds of] is well-known as lectin which should label, for example, ConA, RCA60, RCA120, LCA, PSA and VFA, L-PHA, E-PHA, WGA, PNA, etc. can be illustrated. Refer to; (9349[J.Biol.Chem., 254, and]-9351(1979): Nature, 194, 495 (1962)) fluorescent antibody technique which can also obtain as a commercial item and can also be manufactured according to a conventional method, medical chemistry experiment lecture 4,263-270;Acta.Endocrinol.Suppl., 168, 206 (1972), etc. for the various above-mentioned lectins. Moreover, what is necessary is just the antibody which especially a limit does not have about the antibody which should label, either and recognizes a sugar chain or **** PUCHIDO specifically. By choosing an antibody appropriately, the high labeling antibody of the singularity over the fixed sugar or **** PUCHIDO and an affinity is obtained.

[0020] In the approach of the above (2), the lectin and the antibody which are not labeled are preferably used as matter for association specifically combined with the fixed sugar chain or **** PUCHIDO. Like the lectin and the antibody which are used in the approach of the above (1) as lectin and an antibody and which should label, if a sugar chain or **** PUCHIDO is recognized specifically, it is not limited exceptionally and can choose from various lectins and an antibody.
[0021] In the approach of the above (2), after making the matter for association which is not labeled combine, the marker specifically combined with this matter for association is made to react, and it joins together. Matter, such as a labeling antibody which has compatibility to lectin or an antibody as a marker or an erythrocyte which has sugar which has compatibility to lectin, and polymeric latex, is mentioned here.
[0022] Although the above (1) and the approach of (2) have merits and demerits when both are compared, the approach of the above (2) is excellent the following point. namely, the thing for which purification of the obtained preparation etc. takes time and effort when the labeling lectin or the labeling antibody which can be used is generally limited by the approach of the above (1), and acquisition is difficult and preparation of labeling lectin or a labeling antibody is tried -- in addition, denaturation that the singularity and affinity of lectin or an antibody change may be caused. The labeling antibody which continues broadly by making the matter for association intervene by the approach of the above (2) in contrast with this can be used, and those difficulties do not exist.
[0023] Each of the indicator lectin in the approach of the above (1) or ligation reactions of a labelled antibody, ligation reactions of the matter for association in the approach of the above (2) which is not labeled, and ligation reactions of a marker can be carried out like this usual kind of reaction like the case of immobilization of said sugar chain or **** PUCHIDO. In that case, the various above mentioned solvents which do not have a bad influence on a reaction can be used, and metal ions, such as calcium and manganese, can also be added to the system of reaction by request. Generally the 45 degrees C or less of the above-mentioned reactions are preferably performed by requiring about 1 - 40 hours under an about 4-40-degree C temperature condition.
[0024] The above (1) and (2) In order to both detect the fixed marker finally obtained in law, according to the configuration of solid phase, and optical property, it is suitably performed by measurement of extinction spectrum change, or measurement of radioactivity. For example, when solid phase is a microplate, the plate reader used for general enzyme immunoassay (ELISA) can detect simple.
[0025]
[Example] Although the example of this invention is shown below and this invention is concretely explained to it, this invention is not limited to these examples.
example 1MMAC (methyl vinyl ether / maleic-anhydride copolymer / n-263, molecular weight 41000) the concentration of 5mg/ml -- a hexane -- melting -- this solution -- microtiter plate (Immulon 200, C.A.Greiner and Sohne, GmbH & CoKG, Germany) of 96

holes It put on each well gently for 30 minutes at every [100microl], in addition a room temperature. the 50mM tris [after throwing away supernatant liquid]-maleic-acid buffer solution (pH7.0) It fixed by adding the melted two-fold-serial-dilution train water solution (1.56 to 400 microg/(ml)) of p-aminophenyl galactopyranoside (it used as a simple model of glycopeptide) to 50microl [every] well. After throwing away supernatant liquid and washing 3 times in TBS, it blocked 5% in BSA (bovine serum albumin)/TBS (tris-hydrochloric-acid buffer solution containing 0.15M NaCl) for 1 hour. After throwing away supernatant liquid, the HRP(horseradish par oxidase)-labeling RCA (castor seed seed lectin) which is the labeling lectin which can recognize a galactose specifically was added every [100micro / l], and was put. Furthermore, threw away supernatant liquid, washed in TBS, it was made to color using O-phenylenediamine which is a substrate to HRP, and the absorbance of 490nm was measured.

[0026] it becomes the concentration of 0.1mg/ml about BS3 (screw SURUHOSAKURENIMIJIRU SUBERETO) instead of MMAC in the same experiment as the above -- as -- 5mM phosphate buffer solution (pH5.0) After melting and putting at a room temperature for 2 hours in addition to 100microl [every] well, it compared using what threw away supernatant liquid and carried out the coat of the plate. The result was shown in drawing 1 . Although the concentration of ligand was not seen by 5microg [/ml / less than] concentration and a difference was hardly expected for drawing 1 to see by BS3 and MMAC, the MMAC was far more efficient more than in 5microg/ml, and it turned out that the concentration dependence target is fixed.

[0027] The time amount at the time of fixing example 2 sugar was changed with 1, 3, and 6 or 18 hours, the same experiment as an example 1 was conducted using MMAC, and the effect of fixed time amount was investigated. The result was shown in drawing 2 . as being shown in drawing 2 -- all fixed time amount -- setting -- concentration -- although the anaclitic result was obtained, the balance was reached mostly in about 6 hours.

[0028] The reaction time of example 3HRP-labeling lectin was changed with 15 minutes, 30 minutes, 1 hour, and 2 hours, and the same experiment as an example 1 was conducted using MMAC. The result was shown in drawing 3 . Reaction yield reached the balance in 1 hour.

[0029] The two-fold-serial-dilution train solution (1.56 to 400 microl./(ml)) of p-aminophenyl galactopyranoside was added and fixed on the microtiter plate which carried out the coat of the MMAC like example 4 example 1. After blocking by BSA/TBS 5%, RCA was added every [50micro / l] and it put at the room temperature for 1 hour. Furthermore washing and blocking were performed, the 4% Homo sapiens A mold erythrocyte was 100microl Added, it put for 1 hour, and the erythrocyte was hemolyzed. Detection measured the absorbance of 415nm by the micro reader. The same experiment was conducted to the plate which does not perform an MMAC coat as a comparison. The result was shown in drawing 4 . As compared with what was fixed without what fixed amination sugar through MMAC

minding MMAC, it became clear that detection by subsequent lectin and the erythrocyte can be performed easily.

[0030] p-aminophenyl GARAKUTOHIDORASHIDO was fixed on the microtiter plate which carried out the coat of the MMAC like example 5 example 1. At that time, after changing [ml] the concentration of the sugar to fix in 6.25 to 400 microg /and blocking by BSA/TBS 5%, RCA0.5mg/ml was added every [100micro / l], and it put at the room temperature for 1 hour. Furthermore, washing and blocking were performed, the antibody (an anti-B-SJA-II antibody, 1000 time dilution) to a vegetable mold N-joint sugar chain was added every [100micro / l], and it was made to react at a room temperature for 1 hour. Washing and blocking were performed, the HRP-labeling anti-rabbit IgG antibody (goat) was 100microl Added as a secondary antibody, and it put for 30 minutes at the room temperature. Coloring was performed by the approach which used O-phenylenediamine and measured the absorbance of 490nm. The result was shown in drawing 5 . Consequently, it became clear that amination sugar can detect with sufficient sensibility by the antibody to sugar and its second antibody.

[0031] They are 0.1mM(s) about example 6 castor-seed seed lectin (RCA) 1mg. CaCl2-30mM NaCl-50mM Trypsin which melted to Tris-HCl(pH7.8) 500microl, heated for 10 minutes and was melted to the same buffer solution 100 degrees C (20microg/2microl) In addition, it put at 37 degrees C for 24 hours, and protein was digested. Furthermore, 0.2M PMSF and 0.2M EDTA was 5microl Added, it heated for 10 minutes at 100 degrees C, and the reaction was stopped. What carried out same actuation without adding protein was made into the blank. A part of this digest was added to the C18 opposition HPLC column, and ** PUCHIDO was separated. Using the column (4x150mm) of C18 opposition system, separation performs rate-of-flow 1.0 ml/min, temperature of 40 degrees C, and detection by 220nm, and is 60% from 0.05% trifluoroacetic acid. 2-propanol: Acetonitrile (7:3) It carried out in the straight-line gradient for 60 minutes. The obtained chromatogram was shown in drawing 6 .

[0032] MMAC (1mg/(ml)) melted to the hexane was added to the well of a titer plate every [100micro / l], and it put for 30 minutes at the room temperature. The ** PUCHIDO fraction isolated preparatively is Speed Vac. It is made to dry, pure water is added and they are 50mM(s) about the part. It melted to the tris-maleic-acid buffer solution, was referred to as 50microl, and added to the well which threw away supernatant liquid. It put at 4 degrees C and ** PUCHIDO was fixed for 18 hours. Moreover, BS3 was used for the change of MMAC and same immobilization was performed. It is 5mM phosphate buffer solution (pH5.0) so that it may become the concentration of 0.1mg/ml about BS3. It melted and the coat was carried out to the well of a titer plate at every [50micro / l], in addition 37 degrees C for 2 hours. Furthermore, a part of ** PUCHIDO fraction isolated preparatively was melted to PBS, and it was referred to as 50microl, and, in addition to the well, incubated with BS3. It put at 4 degrees C and ** PUCHIDO was fixed for 18 hours.

[0033] The well of the plate which fixed ** PUCHIDO was washed 3 times in TBS, and it carried out by BSA/TBS 5% and blocked at the room temperature for 1 hour. Supernatant liquid was thrown away, 1000 ********** of the HRP-labeling (concanavalin A) ConA were 100microl Added, and it was made to react at a room temperature for 1 hour. The HRP-labeling ConA combined with ** PUCHIDO containing the sugar after washing and in a well was colored by the approach using O-phenylenediamine, and the absorbance of 490nm was measured and detected. The result was shown in drawing 6 . A positivity and the detected thing are the peaks (29, 31, 44, 47, 47, 48, 51, 54, 58, 59, 60, 65, 67) which described the number by drawing 6 .
[0034] Moreover, the result of having compared the MMAC coat method with BS3 law about these fractions was shown in drawing 7 . Consequently, the fraction (a peak 29, 44, 48, 51, 67) which an absorbance is [to fix by MMAC on the whole] higher, and is hardly detected in BS3 was also detectable in MMAC. ** PUCHIDO which does not attach a sugar chain into the fraction especially was intermingled, and when there were few amounts of **** PUCHIDO (a peak 44 and 54), the difference of the amount of immobilization of MMAC and BS3 was seen. Moreover, when the coat of the front face of a titer plate was not carried out, ** PUCHIDO was not detected at all.
[0035] The Homo sapiens milk oligosaccharide was blocked in constant-rate ****, and 5%BSA/TBS on the microtiter plate which carried out the coat of the MMAC like example 7 example 1. 25microg [/ml] monoclonal antibody (NS10C17) liquid (TBS containing 0.15M NaCl, 10%BSA, and 0.1% NaN3) was added, and it was made to react for 30 minutes at a room temperature. It washed 3 times after the reaction in TBS, the HRP-labeling anti-mouse IgM antibody solution was added as a secondary antibody, and it put for 30 minutes at the room temperature. Coloring was performed by the approach which used O-phenylenediamine, and measured the absorbance of 490nm. Consequently, LNDI (RAKUTO-N including Leb structure [Fucalphal->2Galbetal->3(Fucalphal->4) GlcNAc] - JIFUKOPENTAOSUI [Fucalphal->2Galbetal->3(Fucalphal->4) GlcNAcbetal->3Galbetal->4Glc]) has detected with sufficient sensibility.
[0036] It is 0.1M in some trypsin digests of RCA in example of reference 1 example 6. AcONa-1mM CaCl2-1mMMgCl2-150mM ConA agarose column which equilibrated with the NaCl buffer solution (3ml) It applies and is after washing and 0.1M in 20ml of buffer solutions. It was eluted by 10ml of methyl alpha-mannoside solutions. After condensing this **** PUCHIDO fraction, the chromatogram applied to the C18 opposition HPLC column was shown in drawing 8 . The number was given to seven peaks which were positivities as a result of detecting by the MMAC method like ** PUCHIDO in an example 6.
[0037] When amino acid sequence analysis was performed about seven peak fractions in the example 1 of example of reference 2 reference, and the peak fraction of 12 obtained in the example 6, a result as shown in Table 1 was brought. (The bag alphabetic character showed the array for which the array analyzed in the underline

section in the glycosylation site is understood from a known report as a continuous line.)
In Table 1, the peak number of a left-vertical shaft shows the electropositive peak fraction number obtained by the reversed phase chromatography of an RCA digest, and the right longitudinal shaft shows the electropositive peak fraction number obtained by the affinity chromatography by the ConA agarose column of an RCA digest.
[0038] In Table 1, ** PUCHIDO to which the high mannose mold sugar chain of B-85 of RCA attaches peaks 1, 2, and 4, and peaks 3, 5, 6, and 7 were ** PUCHIDO with the high mannose mold sugar chain of B-135 of RCA. The complex mold sugar chain combined with A-10 and B-73 was not obtained in a ConA agarose column. On the other hand, according to the MMAC coat method, peaks 31, 44, 51, 66, and 67 were equivalent to B-85, A-10, B-135, A-10, and A-10, and were able to detect undetectable **** PUCHIDO by the ConA agarose method, respectively. Therefore, in the ConA agarose column, it became clear by detection of the sugar chain by the MMAC fixed approach that **** PUCHIDO which was not obtained is detectable. In addition, amino acid sequence analysis is Applied Bionsystems. It analyzed in Protein sequencer Model 476A.
[0039]
[Effect of the Invention] According to the approach of this invention, sugar chain structure can be determined that it is simple and efficiently by combining a sugar chain with solid phase in share by analyzing an interaction with the matter which has sugar recognition ability, such as lectin, an antibody, and sucroclastic enzyme. Furthermore, according to this invention approach, the sugar chain which was not detected by the approach of analyzing with the chromatography using the fixed lectin which is a conventional method can also be detected. Moreover, those sugar chain structures can be determined at once about the sample of a minute amount variety. As for the approach of this invention, application in the direction in which the intervention of sugar chains, such as development of a cancer diagnostic drug etc., is expected is expected.
[0040] The method of determining the sugar chain structure of this invention, i.e., the sugar chain of (1) glycoconjugate origin, and glycopeptide, Make the solid phase coated with the polymer which has the reaction radical which reacts with the molecule end group of this sugar chain or this glycopeptide, and can form covalent bond react, and a fixed sugar chain or fixed glycopeptide is generated. This fixed sugar chain or fixed glycopeptide, and the labeled matter that is specifically combined with these are made to react. Or (2-2) this fixed sugar chain or fixed glycopeptide, (2-1) Make the matter for association specifically combined with these react, and the product obtained and the marker specifically combined with this matter for association are made to react further. Subsequently (3) It is as follows when the desirable mode of an approach which determines the sugar chain structure characterized by detecting the labeled solid phase to generate is summarized.

[0041] (i) In the above (1), the sugar chain and **** PUCHIDO which should be fixed in solid phase digest glycoconjugate with an enzyme, and prepare it.
(ii) In the above (1), the reaction radical which can form covalent bond is an epoxy group, a halogen atom, the amino group, a hydrazino radical, a carboxyl group, an acid-anhydride radical, or a formyl group.
(iii) In the above (1), the molecular weight of the polymer which has a reaction radical is 400-1 million.
[0042] (iv) In the above (2-1), the labeled matter is indicator lectin.
(v) In the above (2-1), the labeled matter is a labelled antibody.
(vi) In the above (2-2), it is the lectin or the antibody by which the matter for association is not labeled, and the marker specifically combined with this matter for association is a labeling antibody, an erythrocyte, or polymeric latex.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The effect affect the amount of RCA immobilization of the polymer for coatings in the immobilization to the plate of p-aminophenyl glycoside and this glycoside concentration (it displays also as an absorbance) is shown.
[Drawing 2] The effect affect the amount of RCA immobilization of the fixed time amount in immobilization of p-aminophenyl glycoside and this glycoside concentration (it displays also as an absorbance) is shown.
[Drawing 3] The effect affect the amount of RCA immobilization (it displays also as an absorbance) of the reaction time of the HRP-lectin (RCA) to fixed p-aminophenyl glycoside and this glycoside concentration is shown.
[Drawing 4] In detection by the erythrocyte of the lectin (RCA) combined with p-aminophenyl glycoside, the effect affect the amount of RCA immobilization of the existence of an MMAC coat and this glycoside concentration (it displays also as an absorbance) is shown.
[Drawing 5] In detection of p-aminophenyl glycoside using an antibody, the effect affect the amount of RCA immobilization of this glycoside concentration (it displays also as an absorbance) is shown.
[Drawing 6] The analysis result by the reversed phase high pressure liquid chromatography of the trypsin digest of RCA is shown.
[Drawing 7] The result of having fixed the peptide fraction of the RCA origin in an MMAC coat and three quart of BS is compared and shown.
[Drawing 8] The analysis result by the affinity chromatography by the ConA agarose column of the digest of RCA is shown.
[Table 1]

ピークNo.	保持時間	アミノ酸配列	糖鎖構造	RCA糖鎖結合位置（グリコペプチド）	ピークNo.
29	19.430	QIWDNRT	高マンノース型	B-85	1
31	20.043	IWDNRT	高マンノース型	B-85	
44	23.423	QYPIINFT	複合型	A-10	
47	24.510	WQIWDNRT	高マンノース型	B-85	2
48	24.976	AVSQGWLPTNNT	高マンノース型	B-135	3
51	26.016	LTQTNIYAVSQGWLPTNNT	高マンノース型	B-135	
54	26.883	WQIWDNRT	高マンノース型	B-85	4
58	28.743	AVSQGWLPTNNT	高マンノース型	B-135	5
59	29.030	AVSQGWLPTNNT	高マンノース型	B-135	6
60	29.403	AVSQGWLPTNNT	高マンノース型	B-135	7
65	31.463	QYPIINFT	複合型	A-10	
67	32.103	QYPIINFT	複合型	A-10	

[Translation done.]